BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C12N 15/52, 15/62, 9/10, C12P 19/62, C07C 327/30 — A3

(11) International Publication Number: **WO 00/01838**

(43) International Publication Date: 13 January 2000 (13.01.00)

(21) International Application Number: PCT/US99/15047

(22) International Filing Date: 2 July 1999 (02.07.99)

(30) Priority Data:
60/091,526 2 July 1998 (02.07.98) US
60/091,610 2 July 1998 (02.07.98) US

(71) Applicant: THE BOARD OF REGENTS OF THE LELAND STANFORD JUNIOR UNIVERSITY [US/US]; Suite 350, 900 Welch Road, Palo Alto, CA 94304 (US).

(72) Inventors: KHOSLA, Chaitan; 740 La Para Avenue, Palo Alto, CA 94306 (US). LAU, Janice; Escondido Village #120C, Stanford, CA 94305 (US). POHL, Nicola, L.; 195 Oak Grove Avenue, Menlo Park, CA 94025 (US).

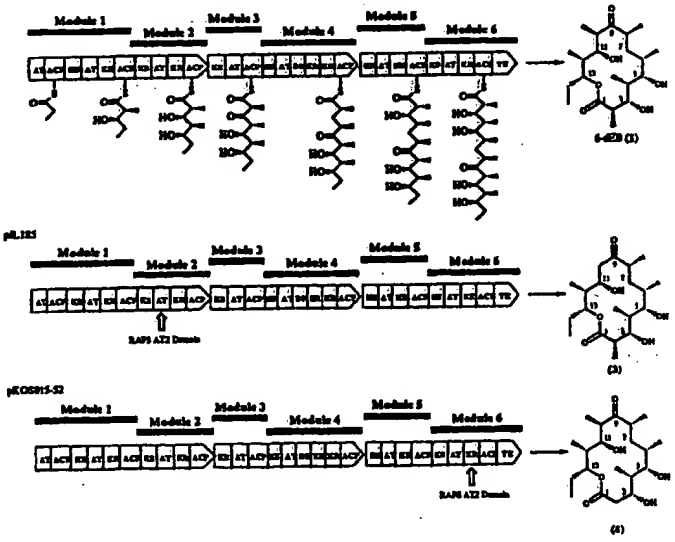
(74) Agents: MURASHIGE, Kate, H. et al.; Morrison & Foerster LLP, 2000 Pennsylvania Avenue, N.W., Washington, DC 20006-1888 (US).

(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(88) Date of publication of the international search report:
16 November 2000 (16.11.00)

(54) Title: METHODS FOR MAKING POLYKETIDES



(57) Abstract

The stereochemical centers of a polyketide can be changed by replacement of ketosynthase domains in the polyketide synthase (PKS) enzyme that produces the polyketide. The specificity of the AT domains of a PKS is determined by a hypervariable region that can be replaced or altered to change the specificity of the AT domain from a naturally occurring extender unit to another naturally or non-naturally occurring extender unit. Non-naturally occurring extender units, including methylmalonyl N-acetyl cysteamine thioester can be incorporated into polyketides in recombinant host cells or in cell-free systems to make polyketides.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

INTERNATIONAL SEARCH REPORT

International Application No
PCT/ S 99/15047

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/52 C12N15/62 C12N9/10 C12P19/62 C07C327/30

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12P C12N C07K C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, PAJ, MEDLINE, BIOSIS, EMBASE, BEILSTEIN Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 01546 A (CORTES JESUS ;LEADLAY PETER F (GB); STAUNTON JAMES (GB); BIOTICA T) 15 January 1998 (1998-01-15) example 32 ---	24-26
X	MILLER W W ET AL.: "N-Acetyl-S-methylmalonylcysteamine, an inhibitor of methylmalonyl coenzyme A isomerase" BIOCHEM BIOPHYS RES COMMUN, vol. 33, no. 4, 25 November 1968 (1968-11-25), pages 569-1573, XP000938495 compound II --- -/--	27,28,30

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 August 2000	20.09.00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	van de Kamp, M

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	RUAN X ET AL.: "Acyltransferase domain substitutions in erythromycin polyketide synthase yield novel erythromycin derivatives" JOURNAL OF BACTERIOLOGY, vol. 179, no. 20, October 1997 (1997-10), pages 6416-6425, XP002131682 cited in the application abstract page 6424, right-hand column, line 41 -page 6425, left-hand column, line 7 ---	1
A	KUHSTOSS S ET AL: "Production of a novel polyketide through the construction of a hybrid polyketide synthase" GENE, vol. 183, no. 1, 1 January 1996 (1996-01-01), pages 231-236, XP004062752 ISSN: 0378-1119 abstract page 235, left-hand column, line 9-30 ---	1
A	STASSI D L ET AL.: "Ethyl-substituted erythromycin derivatives produced by directed metabolic engineering" PROC. NATL. ACAD. SCI. USA, vol. 95, no. 13, 23 June 1998 (1998-06-23), pages 7305-7309, XP002131683 abstract ---	1
A	LEONARD KATZ: "Manipulation of modular polyketide synthases" CHEMICAL REVIEWS, vol. 97, no. 7, 1997, pages 2557-2575, XP002103748 ISSN: 0009-2665 page 2560, paragraph II.B page 2570, paragraph VI.B ---	1
A	KAO C M ET AL.: "Evidence for two catalytically independent clusters of active sites in a functional modular polyketide synthase" BIOCHEMISTRY, vol. 35, no. 38, 24 September 1996 (1996-09-24), pages 12363-12368, XP002131684 abstract --- -/--	1,24

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MARSDEN A F A ET AL.: "Stereospecific acyl transfers on the erythromycin-producing polyketide synthase" SCIENCE, vol. 263, no. 5145, 21 January 1994 (1994-01-21), pages 378-380, XP002131685 abstract page 379, column 2, line 18 -column 3, line 49 ---	24
A	WEISSMAN K J ET AL.: "The molecular basis of Celmer's rules: the stereochemistry of the condensation step in chain extension on the erythromycin polyketide synthase" BIOCHEMISTRY, vol. 36, no. 45, 11 November 1997 (1997-11-11), pages 13849-13855, XP002144574 abstract ---	24
P,X	LAU, JANICE ET AL: "Dissecting and manipulating substrate specificity of the acyltransferase domains of modular polyketide synthases." BOOK OF ABSTRACTS, 216TH ACS NATIONAL MEETING, BOSTON, AUGUST 23-27 (1998), BTEC-028 , 23 August 1998 (1998-08-23), page 28 XP000884730 abstract ---	1-3,5,8, 9
P,X	LAU J ET AL.: "Dissecting the role of acyltransferase domains of modular polyketide synthases in the choice and stereochemical fate of extender units" BIOCHEMISTRY, vol. 38, no. 5, 2 February 1999 (1999-02-02), pages 1643-1651, XP002131687 the whole document page 1649, right-hand column, line 27-31 ---	1-5, 8-10,24
P,X	POHL N L ET AL.: "Synthesis and incorporation of an N-acetylcysteamine analogue of methylmalonyl-CoA by a modular polyketide synthase" JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, vol. 120, no. 43, 20 October 1998 (1998-10-20), pages 11206-11207, XP002146272 compound 2 --- -/--	27,28, 30,31

INTERNATIONAL SEARCH REPORT

International Application No
PCT 3 99/15047

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	BÖHM I ET AL.: "Engineering of a minimal modular polyketide synthase, and targeted alteration of the stereospecificity of polyketide chain extension." CHEMISTRY AND BIOLOGY, vol. 5, no. 8, 6 July 1998 (1998-07-06), pages 407-412, XP000879060 abstract figure 3 page 410, right-hand column, line 3 -page 411, left-hand column, line 22 page 411, left-hand column, line 37-49 ---	24-26
T	KHOSLA C. ET AL: "Tolerance and specificity of polyketide synthases." ANNUAL REVIEW OF BIOCHEMISTRY, vol. 68, 1999, pages 219-253, XP000884453 page 229-231 page 234-241 -----	1-5, 8-10,24

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PC. S 99/15047

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9801546 A	15-01-1998	AU 3450997 A	02-02-1998
		AU 3451497 A	02-02-1998
		BG 103133 A	28-04-2000
		BR 9710209 A	11-01-2000
		CA 2259420 A	15-01-1998
		CA 2259463 A	15-01-1998
		CN 1229438 A	22-09-1999
		EP 0909327 A	21-04-1999
		EP 0910633 A	28-04-1999
		WO 9801571 A	15-01-1998
		GB 2331518 A	26-05-1999
		NO 990012 A	23-02-1999
		PL 331285 A	05-07-1999
		AU 7666198 A	30-12-1998
		EP 0983348 A	08-03-2000
		WO 9854308 A	03-12-1998